REMARKS

Claims 1-7 are currently pending in this Application, Claim 1 has been amended. In view of the following remarks, Applicants respectfully request reconsideration by the Examiner, and advancement of the application to allowance.

Objection to the Specification

Applicants have attached a §1.132 Declaration (See Appendix I) to further clarify the composition of BIODAC® granules. Attached to the Declaration is an MSDS of BIODAC® granules from the effective filing date of the present application.

Rejections under 35 U.S.C. § 102(b)

The Examiner rejects Claims 1-3 and 5 under 35 U.S.C. § 102 as being anticipated by Kelly (U.S. Pat. No. 5,556,631) as evidenced by Bush et al. (U.S. Pat. No. 4,404,339) and the MSDS for glycerol trioleate (Triolein), accessed 5/20/2009 from www.sigma-aldrich.com.

Kelly discloses a "solid pesticide composition such as a bait or granule which is made water resistant by coating the solid pesticide with a hydrophobic fatty acid poly ester of sucrose, sorbitol, sorbinose, glycerol and/or raffinose." (Kelly, Abstract). These water resistant pesticide compositions are prepared by applying the pesticide to any of the traditional solid carriers or substrates and mixing with a high melting solid hydrophobic ester with heat to form a uniform hydrophobic coating on the carrier. (Kelly, Col. 1, Lines 49-54).

Applicants' Claim 1, as amended, discloses a composition of matter useful as a pesticide which comprises, "4-15% by weight of at least one nonionic surfactant." Kelly fails to recite a pesticide composition that comprises a nonionic surfactant. Example 7 of Kelly (Col. 4, Lines 43-50) discloses a Glycerol Trioleate. Examiner argues that Glycerol Trioleate is a known surfactant as evidenced by Bush. Applicants argue that Glycerol Trioleate (Triolein) is not a surfactant, but a fat.

(See U.S. Patent No. 6,006,754, Claim 14 in Appendix II). Surfactants have a hydrophobic part and a hydrophilic part. Rather, Gylcerol Trioleate only has a hydrophobic part. No hydrophilic part is present in Glycerol Trioleate. This goes along with Kelly's teaching of "forming a uniform hydrophobic coating on the carrier" to promote water resistance (Kelly, Col. 1, Lines 53-54). Furthermore, a closer examination of the list presented by Bush et al. reveals his use of the term "surfactant" to be a misnomer. For example, Bush lists, "[a]s examples of suitable examples surfactants there may be named...liquid vegetable and animal fats and oils, and the like." (Bush, Col. 7, Lines 57-63). A surfactant chemist would not refer to vegetable and animal fats and oils as being surfactants. Therefore, Kelly fails to disclose all claimed elements of Applicants' invention. For the above reasons, Kelly fails to anticipate Applicants' invention.

Given that Claims 2-3 and 5 depend from Claim 1, Applicants respectfully submit that Claims 2-3 and 5 are allowable. Accordingly, applicants respectfully request that the Examiner reconsider, withdraw the rejection and allow Claims 1-3 and 5.

Rejections under 35 U.S.C. § 103(a)

The Examiner rejects Claims 4 and 7 under 35 U.S.C. § 103(a) as being unpatentable over Kelly (U.S. Pat. No. 5,556,631) as evidenced by Bush et al. (U.S. Pat. No. 4,404,339) and the MSDS for glycerol trioleate (Triolein), accessed 5/20/2009 from www.sigma-aldrich.com.

For the same reasons argued in the 102(b) rejection above, Kelly as evidenced by Bush and the MSDS for glycerol trioleate fail to recite every element of Applicants' invention. Therefore the cited references cannot render obvious Claims 4 and 7.

Applicants further argue that Kelly does not teach or suggest Applicants' invention. Kelly teaches modifying the surface of the carrier of the pesticide (with a hydrophobic coating) so as to prevent the pesticide from caking/degenerating. (Kelly, Abstract and Col. 1, Lines 23-32). Rather, Applicants' invention focuses on using a surfactant to overcome the affinity/binding of the pesticide to the inert carrier so that the pesticide is released from the inert carrier and enters the environment where it must be to be effective. (Application, Page 3, first and second full paragraph).

If an independent claim is nonobvious under 35 U.S.C. 103, then any claim depending therefrom is nonobviuos. *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988). For the above reasons, Applicants respectfully request that the Examiner remove the 103(a) rejections to Claims 4 and 7.

Rejections under 35 U.S.C. § 103(a)

The Examiner rejects Claims 1 and 5-7 under 35 U.S.C. § 103(a) as being unpatentable over Vrabel et al. (U.S. Pat. No. 6,004,904) as evidenced by the MSDS for RHODAFAC® Re 610 Nonylphenol Polyethoxylate Phosphate Ester (Ashland, updated 1/26/1998) and the MSDS for IGEPAL CA-630 (Octylphenoxy)polyethoxyethathanol (revised 4/2/2003).

Vrabel describes a method for selective control of an unwanted turfgrass or weed species in the presence of a desired turfgrass species at a turfgrass locus by employing an isoxazole compound. (Vrabel, Col. 2, Lines 22-31).

Examiner argues that Vrabel generally teaches that typical pesticide granules can have from 0.5 to 15% surfactant. (Office Action, Mail Date 05/27/2009; Page 8, first line, last paragraph). Applicants argue that Vrabel's teaching is inapplicable because Vrabel is discussing the use of surfactants in a suspension concentrate, which is a liquid-base formulation. This teaching would not apply to the dry granule formulations of Applicants' invention. Applicants' invention is not concerned with the even dispersal of emulsions on the cellulose carrier granules (Office Action, Mail Date 05/27/2009; Page 9, Lines 4-5), rather Applicants are concerned with releasing active ingredients that bind tightly with the granule. (Application, Page 3, first and second full paragraphs). Vrabel specifically teaches the "use of at least one surfactant is generally required because the active ingredients are not water soluble while the spraying vehicle is water. (Column 3, lines 63-65). Vrabel does not teach the use of surfactants to release pesticides from solid, cellulostic granular carrier.

Applicants further argue that Vrabel fails to teach or suggest the use of nonionic surfactants from 4-15%. Vrabel's Example 2 includes 1% IGEPAL CA-630 (Octylphenoxy)-polyethoxyethathanol (surfactant) and 1.0% RHODAFAC® Re 610 Nonylphenol Polyethoxylate Phosphate Ester (surfactant) (Col. 7, Lines 24-33). It should be noted that RHODAFAC® Re 610

Nonylphenol Polyethoxylate Phosphate Ester is an anionic surfactant. Rather, Applicants teach that "[t]esting of the formulations showed that the greatest insect control was provided by the granule formulations containing 6% or more surfactant. (Application, Page 16, first full paragraph).

The prior art must suggest the desirability of the claimed invention. (MPEP 2143.01). Surfactants are traditionally used to bring two immiscible (unblendable) materials together into an emulsion. As taught in Vrabel, the purpose of the surfactant is to act as an emulsifying or wetting agent and the use of at least one surfactant is required because the active ingredients are typically not water soluble. (Vrabel, Col. 3, Lines 55-56). Rather, Applicants discovered that surfactants can be used for the surprising and unexpected result of using a surfactant to overcoming the affinity/binding of the pesticide on the dry carrier materials. (Application, Page 3, second full paragraph). For the above reasons, the cited references do not teach or suggest all of the claimed limitations to support a *prima facia* case of obviousness for Claims 1 and 5-7.

Given that Claims 5-7 depend from allowable Claim 1, Applicants respectfully submit that Claims 5-7 are allowable. Accordingly, applicants respectfully request that the Examiner reconsider, withdraw the rejection and allow Claims 1 and 5-7.

Rejections under 35 U.S.C. § 103(a)

The Examiner rejects Claims 2-4 under 35 U.S.C. § 103(a) as being unpatentable over Vrabel et al. (U.S. Pat. No. 6,004,904) as evidenced by the MSDS for RHODAFAC® Re 610 Nonylphenol Polyethoxylate Phosphate Ester (Ashland, updated 1/26/1998) and the MSDS for IGEPAL CA-630 (Octylphenoxy)polyethoxyethathanol (revised 4/2/2003) in further view of Turnbull (U.S. Patent No. 5,705,516).

As argued in the preceeding section, Claims 2-4 depend from allowable Claim 1, Applicants respectfully request that the Examiner reconsider and withdraw the rejection and allow Claims 2-4.

Rejections under 35 U.S.C. § 103(a)

The Examiner rejects Claims 1-3 and 5-7 under 35 U.S.C. § 103(a) as being unpatentable over Ferrell et al. (U.S. Pat. No. 5,750,130) as evidenced by the MSDS for BRIJ® 72 Polyoxyethylene (2) Stearyl Ether (Sigma-Aldrich, updated 10/2/2007).

Ferrell discloses "[g]ranular pesticide compositions wherein a pesticide material is applied to a granular substrate using a carrier composition which provides improved adhesion of the pesticide to the substrate and abrasion resistance." (Ferrell, Abstract, underlining added for emphasis).

Applicants note that the "carrier materials" listed in Ferrell are comparable to Applicants' nonionic surfactant element and the substrate compositions listed in Ferrell are comparable to Applicants' cellulosic granular carrier element.

Ferrell teaches "carrier materials being characterized by being...nonreactive with the pesticide and substrate composition." (Ferrell, Col. 3, Lines 44-50). Rather, Applicants' invention teaches using the surfactant to "overcome the affinity/binding of the pesticide to the inert carrier. (Application, Page 3, second full paragraph). Therefore, instead of using the carrier composition to provide improved adhesion of the pesticide to the substrate, as in Ferrell, Applicant is using a surfactant to decrease adhesion of the pesticide to the substrate.

Whereas Ferrell teaches the rate of release can be controlled by the amount of water and water solubility of the carrier material, Applicants teach controlling the release rate by modifying the amount of surfactant used. In particular, Ferrell teaches that the "rate at which the pesticides are released when the compositions encounter moist conditions can be controlled by the level of and the water solubility of the surfactant materials added." (Ferrell, Col. 43, Lines 18-23). On the other hand, Applicants teach that "[t]esting of the formulations showed that the greatest insect control was provided by the granule formulations containing 6% or more surfactant. (Application, Page 16, first full paragraph).

Applicants argue that the references fail to teach or suggest all the claimed limitations of the present invention. In particular, the above references, alone or in combination, fail to teach or suggest that an increased use of surfactant, in particular one that uses "4-15% by weight of at least one nonionic surfactant" would increase activity of pesiticides on cellulosic granular carriers.

Given that Claims 2-3 and 5-7 depend from allowable Claim 1, Applicants respectfully submit that Claims 2-3 and 5-7 are allowable. Accordingly, applicants respectfully request that the Examiner reconsider, withdraw the rejection and allow Claims 1-3 and 5-7.

Rejections under 35 U.S.C. § 103(a)

The Examiner rejects Claim 4 under 35 U.S.C. § 103(a) as being unpatentable over Ferrell et al. (U.S. Pat. No. 5,750,130) as evidenced by the MSDS for BRIJ® 72 Polyoxyethylene (2) Stearyl Ether (Sigma-Aldrich, updated 10/2/2007) in further view of Turnbull (U.S. Patent No. 5,705,516).

As argued in the preceeding section, Claim 4 depends from allowable Claim 1, Applicants respectfully request that the Examiner reconsider and withdraw the rejection and allow Claim 4.

CONCLUSION

It is respectfully submitted that the claims now pending in this Application stand in condition for allowance, and that action is respectfully requested. If the Examiner believes a telephonic conversation would aid the prosecution of this Application in any way, a call to the undersigned would be welcome.

Please charge all fees related to this matter to Deposit Account 08-3442.

Dated: 9-12-9

Respectfully submitted,

Edward D. Korompai
Reg. No. 55,344
Attorney for Applicant

APPENDIX I

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Alan J. Stern	§	Confirmation No.: 8841
Serial No.: 10/582,156	§	Art Unit: 1611
Filed: June 08, 2006	§	Examiner: Klinkel, Kortney L.
Title: Surfactant Enhanced Quick Release Pesticide Granules	§	

RULE 1.132 DECLARATION OF ALAN J. STERN

I, Alan J. Stern, the undersigned, state the following:

1. I have a Ph.D. in Organic Chemistry from The Ohio State University.

2. I have been employed in the surfactant/agrochemical industry from 1988 through 2009 and have worked with Huntsman since 2003; my current title is Research Chemist, Agrochemicals Group. My current work still includes efforts to develop new and improved agrochemical formulations that can be used in crop protection and lawn and garden markets.

3. I am an inventor of the subject matter of US Patent Application 10/582,156.

4. I declare that one skilled in the art would know at the time the above identified application was filed that BIODAC® granules are a cellulose complex that is a mixture of paper fiber, kaolin clay, calcium carbonate, and titanium dioxide. Attached to this Declaration as Exhibit I is a Material Data Safety Sheet from 2002/2003 that describes the BIODAC® products.

I declare that all statements made of my own knowledge are true, and that all statement made on information and belief are believed to be true. I made these statements with the knowledge that wilful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and may jeopardize the validity of the application or any patent issued thereon.

August 18, 2009
Date

Alan J. Stern

EXHIBIT I

Material Safety Data Sheet

Following is a replication of the Material Safety Data Sheet (MSDS) for Biodac®

Section I: Material Identification
Section II: Ingredients and Hazards
Section III: Physical Data
Section IV: Fire and Explosion Data
Section V: Health Hazard Information
Section VI: Reactivity Data
Section VII: Spill, Leak and Disposal Procedures
Section VIII: Special Protection Information
Section IX: Special Precautions and Comments

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SECTION I.
MATERIAL IDENTIFICATION

Chemical Name and Synonyms: Cellulose Complex
Trade Name and Synonyms: BIODAC®
Chemical Family: Cellulose Complex

Formula: Chemical Blend

Paper Fiber	CAS# 9004-34-6
Kaolin Clay	CAS# 1332-58-7
Calcium Carbonate	CAS# 471-34-1
Titanium Dioxide	CAS# 13463-67-7

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SECTION II.
INGREDIENTS AND HAZARDS

The materials listed in this Material Safety Data Sheet are classified as non-hazardous in accordance with the definition as set forth in 29 CFR part 1910.1200(g) (2) (c).

EMERGENCY INFORMATION:

EMERGENCY CONTACT: "(800)424-

EMERGENCY CONTACT: (800) 424-9300"
IS TO BE USED "ONLY IN THE EVENT OF CHEMICAL EMERGENCIES INVOLVING A SPILL, LEAK, FIRE, EXPOSURE, OR ACCIDENTS INVOLVING CHEMICALS." AVAILABLE 24 HOURS.

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SECTION III.
PHYSICAL DATA

Boiling Point at 1 atm, deg F:	N/A Solid
Vapor Pressure at (mm Hg):	N/A Solid
Vapor Density (air=1):	N/A Solid
Water Solubility:	N/A Solid
Appearance and Odor:	Typically gray, with musty odor.
Evap. Rate (_____=):	N/A
Volatiles, % by Volume:	<1
Molecular Weight:	N/A
Bulk Density:	38-46 lbs/cft.

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SECTION IV.
FIRE AND EXPLOSION DATA

Flammability Limits in Air:	LOWER N/A UPPER N/A
Extinguishing Media:	Water, CO2, Foam
Special Fire Fighting Procedures:	None Required
Unusual Fire and Explosion Hazards:	None
Autoignition Temp:	220°C
TLV:	None Established

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SECTION V.
HEALTH HAZARD INFORMATION

FIRST AID

Eye Contact:	Flush with water or eye drops to remove particles.
Skin Contact:	Wash with soap and water.
Inhalation:	Remove by expiration.
Ingestion:	Induce vomiting.

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SECTION VI.
REACTIVITY DATA

Stability:	Stable:	Conditions to avoid: None Known
Incompatibility (Materials to Avoid):	None	
Hazardous Decomposition:	None	
Hazardous Polymerization:	Will Not Occur:	Conditions to avoid: None Known

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SECTION VII.
SPILL, LEAK, AND DISPOSAL PROCEDURES

Spills, Leaks:	Sweep up and contain for disposal
Waste Disposal Method:	Incinerate or bury in an approved landfill in compliance with all federal, state, and local laws.

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SECTION VIII.
SPECIAL PROTECTION INFORMATION

Respiratory Protection (Specify Type):	None Required
Ventilation:	Use local exhaust to control possible air-borne material.
Other:	None
Protective Gloves:	Gloves recommended for prolonged exposure.
Eye Protection:	Eyeglasses with side shields recommended.

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SECTION IX.
SPECIAL PRECAUTIONS AND COMMENTS

Storage & Handling Information:	Store in a dry location away from foodstuffs and animal feed.
Other Precautions:	None
DOT Class:	Cellulose Granular

Biodac® | PaPurr® | Gran-sorb® | About Us | Contact | Home

APPENDIX II

US006006754A

United States Patent [19]

Janghorbani et al.

[11] Patent Number: 6,006,754

[45] Date of Patent: Dec. 28, 1999

[54] **METHOD FOR MEASURING FAT DIGESTION AND ABSORPTION FORMULATION TO AID IN MEASURING FAT ABSORPTION**

[75] Inventors: **Morteza X. Janghorbani**, Chicago, Ill.; **Sally A. Schuette**, Crown Point, Ind.; **Mitchell B. Cohen**, Cincinnati, Ohio

[73] Assignee: **Biochem Analysis Corporation**, Chicago, Ill.

[21] Appl. No.: **09/005,305**

[22] Filed: **Jan. 9, 1998**

[51] **Int. Cl.**[6] **A61B 19/00**

[52] **U.S. Cl.** **128/898**; 424/94.21; 424/690

[58] **Field of Search** 424/690, 1, 94.21; 436/86

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,826,679	5/1989	Roy	424/94.21
4,840,795	6/1989	Ben-Sasson	424/690

OTHER PUBLICATIONS

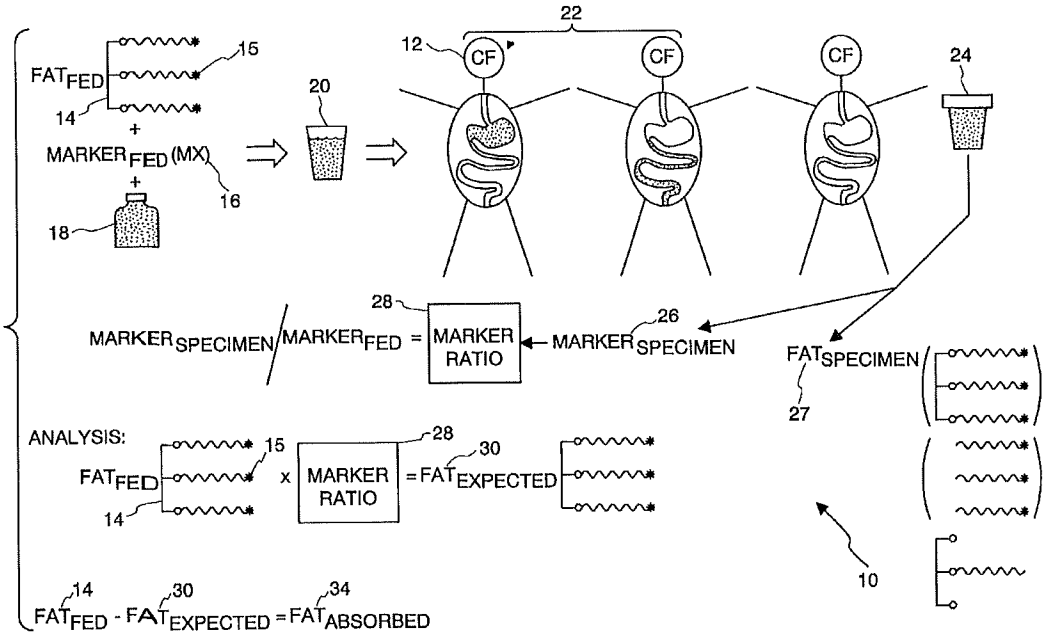
Schuette et al. Journal of the American College of Nutrition, vol. 12, No. 3, pp. 307–315 (1993) "Dysprosium as a Non–absorbable Marker for Studies of Mineral Absorption with Stable Isotope Tracers in Human Subjects".

Primary Examiner—Max Hindenburg
Assistant Examiner—Brian Szmal
Attorney, Agent, or Firm—Cherskov & Flaynik

[57] **ABSTRACT**

A method for measuring fat assimilation, such as fat digestion and fat absorption, in a person is provided comprising feeding the person labeled fat, nonabsorbable marker, and a means for coloring stool; allowing the fat, marker and stool coloring means to travel through the digestive tract of the person; monitoring stool from the person for the appearance of the coloring means; collecting stool containing the coloring means; and measuring the amount of marker and labeled fat in the stool to determine the portion of fat digested and/or absorbed by the system. Also provided is a formulation to facilitate one-step administration and specimen collection of a fat-digestion and fat-absorption determinant.

19 Claims, 2 Drawing Sheets



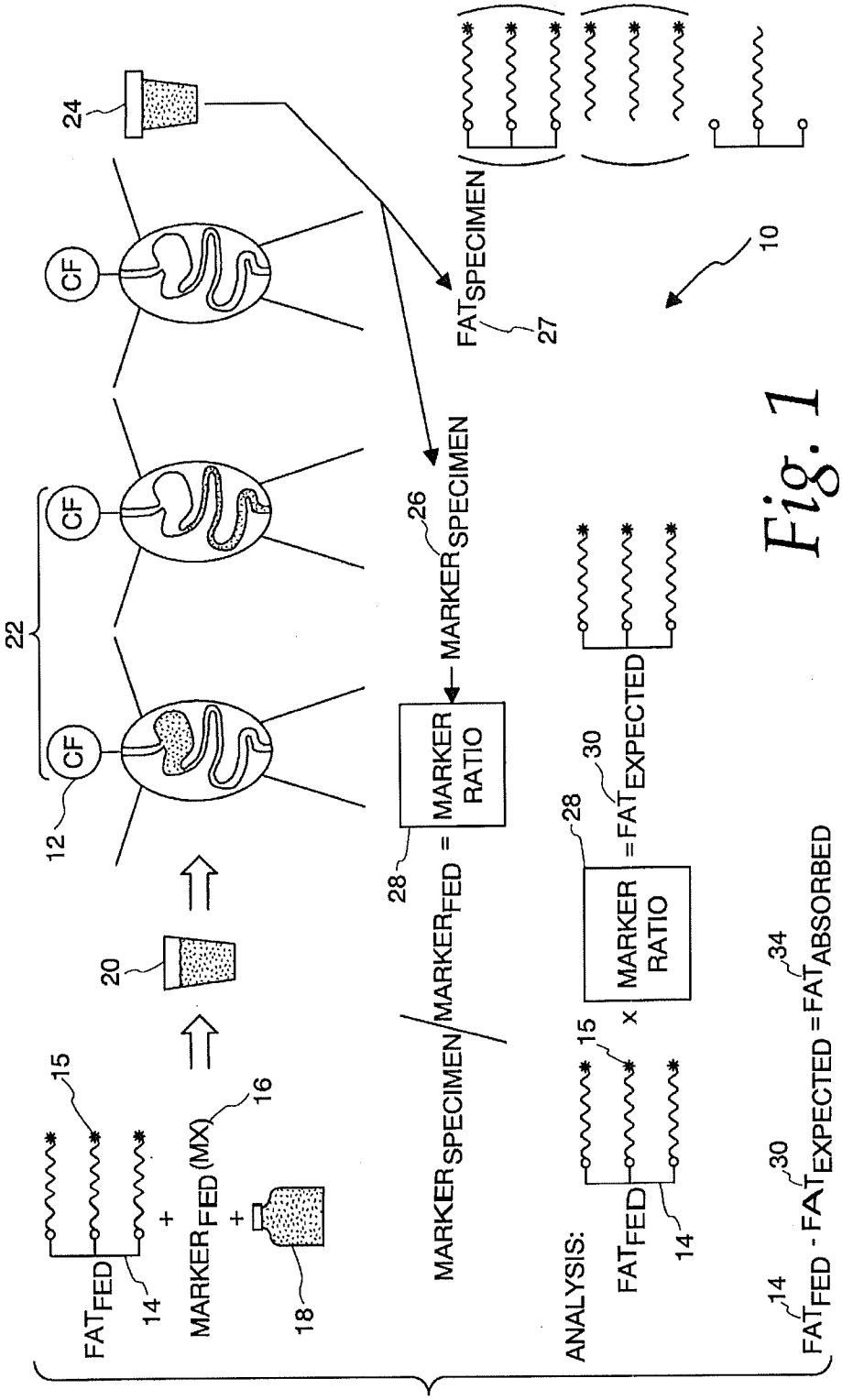
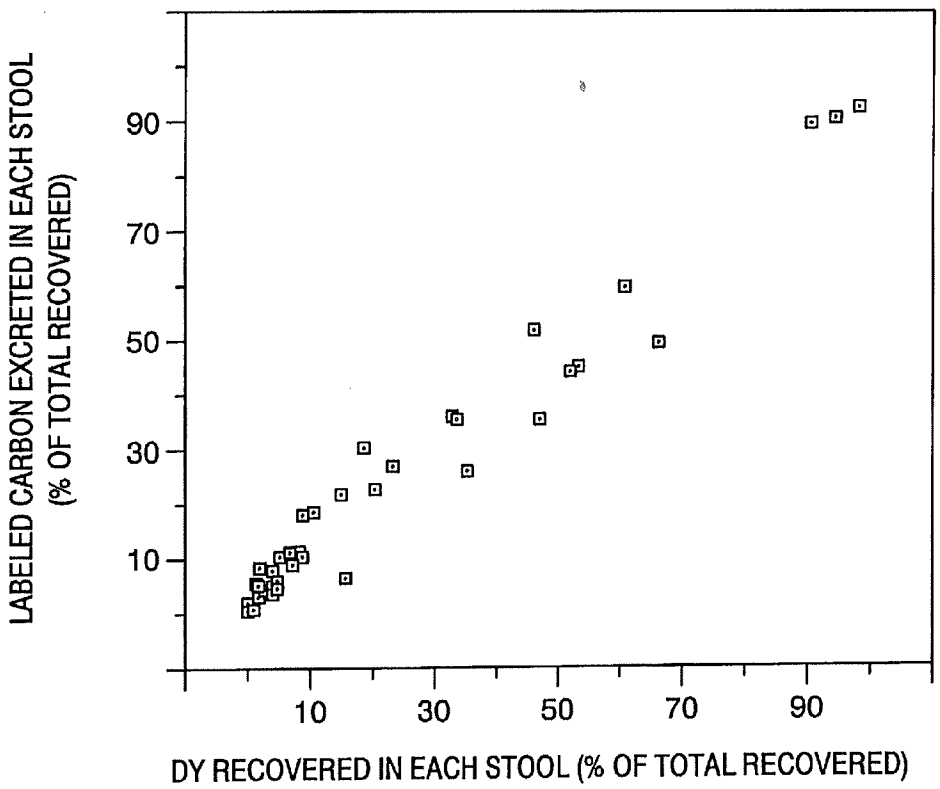

FAT FED
14
+
MARKER FED (MX)
16
+
18
15
20
22
CF
CF
CF
12
24
MARKER SPECIMEN
MARKER FED =
28
MARKER RATIO
26
MARKER SPECIMEN
FAT SPECIMEN
27
10
ANALYSIS:
FAT FED
14
15
x
28
MARKER RATIO
= FAT EXPECTED
30
14
FAT FED - FAT EXPECTED = FAT ABSORBED
30
34

Fig. 1

Fig. 2


LABELED CARBON EXCRETED IN EACH STOOL
(% OF TOTAL RECOVERED)
90
70
50
30
10
10
30
50
70
90
DY RECOVERED IN EACH STOOL (% OF TOTAL RECOVERED)

METHOD FOR MEASURING FAT DIGESTION AND ABSORPTION FORMULATION TO AID IN MEASURING FAT ABSORPTION

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under Contract Nos. R-44-DK-48190 and R-42-DK-448537 between the National Institutes of Health and BioChem Analysis Corporation. The United States Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to a method and formulation for measuring fat digestion and absorption and more specifically to a method and formulation for measuring fat digestion and absorption in individuals predisposed to inadequate fat digestion and absorption due to disease.

2. Background of the Invention

Certain diseases prevent persons from properly digesting and/or absorbing fat (triglycerides). For example, cystic fibrosis, the most common lethal genetic disorder of Caucasian populations, often results in a patient's inability to digest fat. This is due to the patient's pancreas being unable to supply the enzymes in sufficient amounts for hydrolysis. Another example is Celiac disease in which absorption of digested dietary fat is impaired due to reduced surface area of the small intestine.

Major advances in cystic fibrosis treatment have increased the life expectancy of patients with cystic fibrosis to more than 30 years. As no treatment is currently on the horizon to alleviate the pancreatic/gastrointestinal components of the disease, there is a growing population of cystic fibrosis patients who require life-long nutritional management. The diets of these patients generally are supplemented with pancreatic enzymes. To assure proper enzyme dosage, however, an accurate measurement of a patient's fat digestion and absorption is required.

A number of methods for determining the extent of fat digestion exist. For example, a direct and somewhat unpleasant method involves duodenal intubation and subsequent measurement of enzyme levels withdrawn from the intestine. The procedure is highly invasive, specialized, expensive and difficult to use in young children.

A myriad of indirect measurement procedures based on the appearance of a test substance in bodily fluids such as blood, urine, or breath are also available. These procedures have drawbacks, such as limitations related to the sampling compartment because of the underlying disease, and complications due to disease of related effects on other organ systems, such as the liver and the kidneys, which can lead to inaccurate results.

As an example of the limitations of current fat digestion and absorption measurement techniques, the problems inherent in the ^{13}C-labeled fat breath test are highlighted below:

1.) Conversion rate of absorbed label to CO_2 is influenced by the metabolic status of the individual. (e.g. liver function);

2.) CO_2 excretion is effected by lung function;

3.) Variations in body fat content influence the rate of CO_2 excretion;

4.) Breath samples are not easily obtained from infants and young children; and

5.) Long (6 to 12 hours) breath sampling times are required.

There are analogous limitations for tests which rely on the appearance of a test substance in blood or urine. Generally, appearance methods are unsuitable for quantitative measurements of gastrointestinal absorption of fat.

A widely used method for measuring fat absorption is the "72-hour stool fat test" (72-h SFT). This test requires that a patient consume 100 grams of fat daily for at least three days, during which the stools are collected for measurement of total fat content. The 72-h SFT is considered the "Gold Standard" for the purpose of determining a patient's ability to digest and/or absorb dietary fat.

However, there are many limitations to the 72-h SFT method. Overall, it is tedious and difficult to perform accurately. For example, a constant fat intake for at least three days is required. Also, quantitative collection of stools, (typically by the patient) is not assured. Only the most diligent adult patients supply accurate information regarding fat intake and accurate stool collection. Hospitalization and close monitoring is required for other adult patients, infants and children, which makes the procedure expensive. As a result, the 72 h SFT is most reliable to detect only significant steatorrhea (excessive fat in stool). Similarly, variability in the coefficient of fat absorption from test to test may be large.

A need exists in the art for an easy and economical method and formulation for directly measuring fat digestion and/or absorption under conditions of normal dietary intake. The method and formulation should depend neither on a patient's skill in collecting all stool for several days, nor in assuring constant fat intake for such a long period of time. The new method should be accurate, reliable, reproducible and noninvasive so as to facilitate easy monitoring and medical management of underlying disease. The results of the new method and formulation would be particularly useful in repetitive monitoring of changes in fat digestion as is recommended for patients with cystic fibrosis.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a method and formulation for measuring fat digestion and/or absorption which overcomes many of the disadvantages of the prior art.

Another object of the present invention to provide a simple method for accurately measuring fat digestion and/or absorption in patients. A feature of the invention is the patient intake of a single formulation, in the form of a tablet or similar medium, and the subsequent collection of a small amount of stool from a single bowel movement. An advantage of the invention is its administration during normal dietary intake, therefore obviating the need for constant fat intake and obviating the need to collect all stools from a patient. Another advantage of the invention is its ability to detect fat malabsorption of clinical importance before some clinical symptoms occur.

Yet another object of the present invention is a convenient method for collecting stool for subsequent analysis of fat absorption, particularly in cystic fibrosis patients. A feature of the method is the use of stool colorant. An advantage of the method is a one-time collection of stool containing the colorant for subsequent analysis. Another advantage is that the results of the method are highly reproducible.

Still another object of the present invention is to provide a formulation for use in determining fat maldigestion and fat

malabsorption. A feature of the formulation is the combination of an isotope-labeled triglyceride, a nonabsorbable fecal marker and a colorant to provide an easy-to-dispense form for self administration. An advantage of using this formulation is the noninvasive and one-step administration and collection method associated with its use to determine levels of fat digestion and absorption in patients.

In brief, a method for measuring fat digestion and/or absorption in a person is provided comprising feeding the person a specific amount of labeled fat, a specific amount of a nonabsorbable marker, and a means for coloring stool (e.g., a visual marker); allowing the fat, the nonabsorbable marker and the stool coloring means to travel through the digestive tract of the person; monitoring stool from the person for the appearance of the coloring means; collecting stool containing the coloring means; and measuring the amount of nonabsorbable marker and labeled fat in a small amount of the colored stool to determine the portion of fat digested and/or absorbed by the person.

Also provided is a formulation which when ingested by a person and later partially retrieved from the digestive system of the person can aid in determining fat digestion and/or absorption characteristics of the person, the formulation comprising a labeled fat; a marker which has the same absorption kinetics as said fat; and a dye mixed with said fat and said marker.

BRIEF DESCRIPTION OF THE DRAWING

The present invention together with the above and other objects and advantages may best be understood from the following detailed description of the embodiment of the invention illustrated in the drawing, wherein:

FIG. **1** is a flow diagram of an exemplary process illustrating the present invention; and

FIG. **2** is a graph comparing the excretion kinetics of lanthanide with ^{13}C in the digestive system, in accordance with features of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The invented method provides a reliable, highly accurate test for fat digestion and absorption that can be self-administered. The method has broad utility which includes the following:

1.) Aids in evaluating persons for the presence or absence of fat maldigestion and malabsorption, even when no clinical signs of the underlying disorder are present;

2.) Assists in monitoring the course of disorders underlying maldigestion or malabsorption; and.

3.) Helps clinicians optimize therapy for individual patients.

The method is based on two principles. First, that a triglyceride representative of dietary neutral fats in the human diet can be labeled with a non-radioactive isotope (any stable isotope such as ^{13}C is suitable) so that its residual presence in stool during several days after its administration quantitatively reflects the residual dietary fat consumed during the same period. Second, that a trace amount of nonabsorbable salt, such as chloride or sulfide salts containing an element or elements from the lanthanide group, can be used as a nonabsorbable marker. When this marker is administered simultaneously, it is completely nonabsorbed by the person and evacuated in feces during the same period of evacuation of the labeled fat.

The inventors have found that certain lanthanide salts (e.g. $DyCl_3$) are quantitatively excreted by patients and follow the same excretion kinetics as labeled triglycerides. Thus, when a known amount of both the marker and labeled fat are consumed with any meal, subsequent chemical analysis of both the labeled fat and lanthanide in any sample of stool containing them permits accurate and rapid measurement of the fat absorbed.

An additional feature of the method is the simultaneous administration of a fecal colorant with the nonabsorbable marker and labeled fat. This obviates the need for collecting all stools subsequent to fat administration. Instead, only a portion of the stool showing the presence of colorant need be collected for subsequent analysis.

An exemplary method is depicted generally in FIG. **1** as numeral **10**. As an initial step, a specific amount of suitable fat **14**, labeled with an isotope **15**, is prepared in a specific amount (FAT_{fed}).

Prior to feeding to the patient, the fat is mixed with a specific amount ($Marker_{fed}$) of nonabsorbable marker **16** and a stool dye **18** to create a mixture **20**. The mixture is administered to the patient **12** and allowed to be metabolized by the patient in the normal course of digestion **22**. The patient monitors stool color for appearance of the dye **18** and selects a stool specimen **24** which indicates the presence of the dye.

The specimen is analyzed for the amount of nonabsorbable Marker ($Marker_{specimen}$) **26** and labeled fat ($Fat_{specimen}$) **27**. The ratio of $Marker_{specimen}$ to $Marker_{fed}$ is used as a multiplier **28** to determine the amount of fat expected **30** to be excreted if all stools evacuated for several days had been collected and analyzed completely ($Fat_{expected}$). As a last step, the amount of fat so determined ($Fat_{expected}$) is subtracted from (Fat_{fed}) to obtain the amount of labeled fat absorbed **34** by the patient **12**.

A salient feature of the above stated method is that a single stool sample will yield accurate and reproducible data regarding maldigestion and/or malabsorption of fat by the patient.

The above procedure is easily followed by providing patients with test kits containing all ingredients premixed. Such premixed entities can take the form of a tablet, a liquid, a powder or similar medium. All isotopic and marker analysis of specimens are performed by persons of ordinary analytical skill in typically equipped isotope-handling laboratories.

Fat Dose- and Chemical Forms and Label Position Detail

Dietary fat consists primarily of triglycerides with a majority of these being of mixed fatty acid composition. Fatty acids differ from each other in their chain-length and degree of unsaturation. Fatty acids in the human diet include the following: octanoic acid (8:0), myristic acid (C12:0), palmitic acid (C16:0), stearic acid (C18:0); oleic acid (C18:1), and linoleic acid (C18:2). The last four fatty acids are the most common fatty acids of the various triglycerides present in animal fats and vegetable oil found in human diets.

In the present invention, ^{13}C-label is incorporated in the fatty acid portion of a selected triglyceride. Inasmuch as a majority of dietary triglycerides are of mixed fatty acid composition, suitable triglycerides also include the mixed variety. Therefore, a myriad of triglycerides, are suitable fat candidates, including but not limited to 1,3-di-stearoyl2 [^{13}C] octanoyl glycerol, 1,3-dioleoyl2[^{13}C] octanoyl glycerol, triolein, tripalmitin, trioctanoin, and combinations thereof. Three ^{13}C-labeled triglycerides are commercially available through Cambridge Isotopes Laboratories, Cambridge, Mass.: triolein, tripalmitin, and trioctanoin.

The inventors have found that the chain-length, degree of saturation and position of labels on the fatty acids of selected

triglycerides determine the sensitivity and specificity of the invented method and formulation to various disorders of fat maldigestion and fat malabsorption. For example, if ^{13}C-palmitic acid is incorporated in positions 1 and 3 (sn-1,3) of the triglyceride, the resulting labeled triglyceride is sensitive to both maldigestion (wherein pancreatic enzymes cleave at positions 1 and 3) and malabsorption. Such a triglyceride is ideal to measure generalized steatorrhea due to different mechanisms.

Alternatively, if ^{13}C is incorporated at position 2 (sn-2) of the triglyceride in the form of ^{13}C-octanoic acid (8:0), the resulting customized triglyceride is not sensitive to fat malabsorption disorders such as celiac disease inasmuch as hydrolysis of the triglyceride releases the ^{13}C-label as 2-monoglyceride. 2-monoglyceride with octanoic acid is highly soluble, making it easily absorbable and therefore not affected by celiac disease. However, this triglyceride is sensitive to disorders of the pancreas that cause pancreatic insufficiency (e.g. cystic fibrosis). Furthermore, the chain length and degree of unsaturation of the unlabeled fatty acid incorporated at positions 1 and 3 further affect the degree of pancreatic insufficiency. For example, 1,3-distearin (18:0) is more sensitive to pancreatic insufficiency than 1,3-diolein (18:1).

The minimum dose of ^{13}C-labeled triglyceride required is estimated based on the following rationale: The ^{13}C-labeled tripalmitin used by the inventors contains 48.1 mg of excess ^{13}C per gram, that is, 48.1 mg of ^{13}C in excess of that due to carbon of natural isotopic composition. If an average absorption of 50 percent is assumed, then total fecal excretion of the excess ^{13}C should be roughly 24 mg for a 1 gram dose. A stool containing 1 percent of the excreted label would, thus, contain 0.24 mg of ^{13}C in excess. The routinely achieved measurement precision for ^{13}C measurements (atom %) in fecal samples is approximately 0.1 percent (RSD). For a daily stool output of 10 grams total carbon (100 mg ^{13}C), 0.1 percent precision corresponds to 0.1 mg of background ^{13}C. Administration of 1 gram of ^{13}C-labeled tripalmitin, under these assumed conditions, would result in a ^{13}C excess content for stools containing 1 percent of the unabsorbed label of 0.245 mg, which corresponds to about 2 σ (two standard deviations) of the measurement precision. Thus, the dose chosen is 1 g ^{13}C-labeled tripalmitin for an adult. Assuming an average body weight of 50 kilograms, this corresponds to 20 mg/kg, which corresponds to the dose reported in the literature for breath tests.

Similar estimates are made when using other ^{13}C-labeled triglycerides, depending on the specifics of the ^{13}C enrichment.

Marker Dose- and Chemical-Forms Detail

Generally, the invented method and formulation provides a detection level of one percent of the amount of nonabsorbable marker that is fed to a patient. As such, dose levels for the marker are chosen so that one percent of the dose excreted in any stool corresponds to 2 σ of a marker's daily background. Previous research by one of the inventors has determined that the expected background content of lanthanides (such as dysprosium) in feces should be 0–10 μg per day (μg/day), given an average value of 3.4±7.8[2σ] obtained from the analysis of 15 daily collections from four adults. Thus, for an average adult, the dose of lanthanide marker is approximately 1 mg. Generally, lanthanide marker doses of between 20 and 50 μg/kg body weight provide good results.

Exemplary Protocol

The following protocol was carried out on nine cystic fibrosis patients in a medical center. Each patient received a single dose of ^{13}C-palmltin (0.700 g) mixed with peanut butter and Dy (1.013 mg) added to milk. Both compounds were fed as part of a meal. Individual stools were collected for five days. If analysis is not performed immediately, the specimens can be frozen.

Each stool was transferred to a tared plastic container and weighed accurately. When thawed, the stool is homogenized and a weighed fraction taken for analysis. The fraction was gently heated to approximately 100° C. until dry (12–24 hours). Each dried sample was ground to a fine powder from which weighed subsamples were taken for measurement of ^{13}C-excess and Dy. All carbon isotopic analyses were performed with an Europa Scientific 20/20 isotope ratio mass spectrometer equipped with Automated Nitrogen Carbon Analyzer (ANCA). All analyses of Dy were carried out with neutron activation analysis. A detailed protocol for Dy analysis is disclosed in Schuette, S.A. et al. Dysprosium as a non-absorbable fecal marker for studies of mineral absorption with stable isotope tracers in human subjects. J Am Coll. Nutr. 12:pp 307–15, 1993, and incorporated herein by reference.

Dy content of each stool was calculated from the results of Dy measurements performed on the stool's subsample, the dry/wet ratio of each stool, and the total wet weight of each stool. The resulting data were expressed as percent of Dy intake present in each stool. From analysis of ^{13}C (atom percent) and total carbon performed for each subsample, total ^{13}C-excess excreted in each stool was calculated according to the following equations:

$$R=[13.0033551(\text{atom \% } ^{13}C)]/12.000000(100\text{-atom \% } ^{13}C)] \quad \text{Eq.1}$$

$$^{13}C^{*}_{stool}=(\{R+RR^{0}-R^{*}-R^{*}R\}/[RR^{0}-R^{*}+RR^{*}])(\text{Total C}) \quad \text{Eq.2}$$

where $^{13}C^{*}_{stool}$=^{13}C-excess in each stool; and where R, R*, and R^0 are $^{13}C/^{12}C$ ratios (wt/wt) for fecal sample of interest, labeled tripalmitin, and baseline fecal sample, respectively. Values of R are calculated from the measured atom percent values using Equation 1.

The results obtained by the inventors indicate that lanthanide salts (e.g. $DyCl_3$) are nonabsorbable markers in human digestive processes. The results also indicate that ^{13}C-excess in each stool (expressed as the fraction of total recovered in all stools for any patient) is the same as its corresponding Dy (also expressed as a fraction of the total recovered).

Table 1 below discloses the correlation between lanthanide recovered and excess ^{13}C recovered. Recoveries of Dy were 93.6 percent or greater in eight of the nine patients with a mean (±1σ) of 108 (±9.9) percent. In these patients, excretion of ^{13}C-label was ≧67.9 percent of the dose. In contrast Dy recovery for CF1 was only 29.5 percent of the dose, probably the result of incomplete collection of stool.

TABLE 1

Recoveries of Dy and ^{13}C-excess in cystic fibrosis patients.

Patient Code	Dy Recovered in all stools (% dose)	^{13}C-Excess recovered in all stools (% dose)
CF#1	29.5	28.8
CF#2	106.7	72.9
CF#3	114.9	93.3
CF#4	93.6	69.3
CF#5	101.8	67.9
CF#6	118.5	107.2
CF#7	116.3	96.2

TABLE 1-continued

Recoveries of Dy and ^{13}C-excess in cystic fibrosis patients.

Patient Code	Dy Recovered in all stools (% dose)	^{13}C-Excess recovered in all stools (% dose)
CF#8	118.9	79.6
CF#9	94.2	80.6

FIG. **2** illustrates the equivalency of the excretion kinetics of Dy and ^{13}C-labeled triglyceride. The graph depicted in FIG. **2** plots ^{13}C-excess in each stool against its Dy content, for all stools obtained from the nine patients. The correlation between Dy and isotope kinetics, as depicted in the data discussed supra, allows for an accurate determination of fat assimilation to be made when just one stool is collected, the only caveat being that the stool must contain both Dy and the isotope. To isolate the specifically enriched stool, a stool colorant is thoroughly mixed with the Dy/isotope cocktail prior to ingestion. Subsequent to ingestion, stools are monitored for appearance of the colorant. Upon appearance of the colored stool, the stool is isolated and used for analysis.

Various colorants are available for use in this one-stool retrieval method, including but not limited to, carmine red and brilliant blue. The colorant is added in an approximate 1.0 mg/kg weight ratio of colorant to patient body weight.

While the invention has been described with reference to details of the illustrated embodiment, these details are not intended to limit the scope of the invention as defined in the appended claims.

The embodiment of the invention in which an exclusive property or privilege is claimed is defined as follows:

What is claimed is:

1. A method for measuring fat assimilation in a person comprising:

a) feeding the person labeled fat, nonabsorbable marker, and a means for coloring stool;

b) allowing the labeled fat, marker and stool coloring means to travel through the digestive tract of the person;

c) monitoring stool from the person for the appearance of the coloring means;

d) collecting stool containing the coloring means; and

e) measuring the amount of marker and labeled fat in the stool to determine the portion of fat assimilated by the person.

2. The method as recited in claim **1** wherein the step of measuring the amount of marker and labeled fat in the stool further comprises:

a) comparing the amount of marker measured in one stool to the specific amount of marker fed to the person to determine the ratio of measured marker to fed marker; and

b) comparing the proportion of measured marker to fed marker with the ratio of measured labeled fat to fed labeled fat to determine the amount of fed labeled fat absorbed by the person.

3. The method as recited in claim **1** wherein the step of collecting stool comprises isolating one stool specimen.

4. The method as recited in claim **1** wherein the labeled fat is selected from the group consisting of 1,3-distearyl, 2[^{13}C] octanoyl glycerol, 1,3-dioleyl, 2[^{13}C] octanoyl glycerol, triolein, tripalmitin, trioctanoin, and combinations thereof.

5. The method as recited in claim **1** wherein the person is fed the labeled fat, marker and colorant simultaneously.

6. The method as recited in claim **1** wherein the nonabsorbable marker contains a lanthanide element.

7. The method as recited in claim **1** wherein the nonabsorbable marker is a salt of a lanthanide element.

8. The method as recited in claim **1** wherein the coloring means is selected from the group consisting of carmine red and brilliant blue.

9. The method as recited in claim **1** wherein the label is an isotope selected from the group consisting of carbon-13, and deuterium.

10. A formulation which when ingested by a person and later partially retrieved from the digestive system of the person can aid in determining fat assimilation characteristics of the person, comprising:

a) a labeled fat;

b) a marker which has the same absorption kinetics as said labeled fat; and

c) a dye mixed with said labeled fat and said marker.

11. The formulation as recited in claim **10** wherein the labeled fat is present in a fat to person weight ratio of approximately 20 mg/kg.

12. The formulation as recited in claim **10** wherein the marker is present in a marker to person weight ratio of approximately 20 μg/kg.

13. The formulation as recited in claim **10** where said labeled fat is a neutral fat.

14. The formulation as recited in claim **10** wherein said labeled fat is selected from the group consisting of 1,3-distearyl, 2[^{13}C] octanoyl glycerol, 1,3-dioloeyl, 2[^{13}C] glycerol, triolein, tripalmitin, trioctanoin, and combinations thereof.

15. The formulation as recited in claim **10** wherein said marker is not absorbed by the person.

16. The formulation has recited in claim **10** wherein the marker is a salt of a lanthanide element.

17. The formulation as recited in claim **10** wherein the marker is a chloride salt of a lanthanide element.

18. The formulation as recited in claim **10** wherein the dye is selected from the group consisting of carmine red and brilliant blue.

19. The formulation as recited in claim **10** wherein the dye is present in a weight ratio to the patient of approximately 1.0 mg/kg.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,006,754
DATED : December 28, 1999
INVENTOR(S) : Janghorbani, et al.

It is certified that error appears in the above-indentified patent and that said Letters Patent is hereby corrected as shown below:

Title page, Item [73], please insert --Children's Hospital Research Foundation, Chicago, Ill.-- as the second Assignee.

Signed and Sealed this

Twenty-sixth Day of December, 2000

Attest:

Q. TODD DICKINSON

Attesting Officer

Director of Patents and Trademarks

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,006, 754

DATED : December 28, 1999

INVENTOR(S) : Janghorbani et al

It is certified that error appears in the above-indentified patent and that said Letters Patent is hereby corrected as shown below:

Item [73], please insert --Children's Hospital Medical Center, Cincinnati, OH--as the second Assignee.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,006,754
DATED : December 28, 1999
INVENTOR(S) : Jamshidani et al.

It is certified that error appears in the above-indentified patent and that said Letters Patent is hereby corrected as shown below:

Claim 7, column 8, line 14, after "a", first occurrence, insert --chloride--.

Claim 16, column 8, line 44, after the word "formulation" delete "has" and insert the [illegible].

Signed and Sealed this

Twenty-fourth Day of April, 2001

Attest:

Nicholas P. Godici

NICHOLAS P. GODICI

Attesting Officer

Acting Director of the United States Patent and Trademark Office